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FLAVONES AND DITERPENES OF *PAMBURUS MISSIONIS* (RUTACEAE)*

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Abstract—A new flavone and two diterpenes have been isolated from the extracts of *Pamburus missionis*. The flavone was identified as an isopentylated apigenin derivative on the basis of spectroscopic studies on it and its diacetate. One of the diterpenes has been identified as daniellic acid and the second as a closely related butenolide.

A previous paper from this laboratory reported [1] the isolation of severine palmitate [2] from the fruit of *Pamburus missionis* (Wt.) Swingle (*Atalantia missionis*), a monotypic genus native to S. India [3]. This paper reports a study on the foliage of this same species.

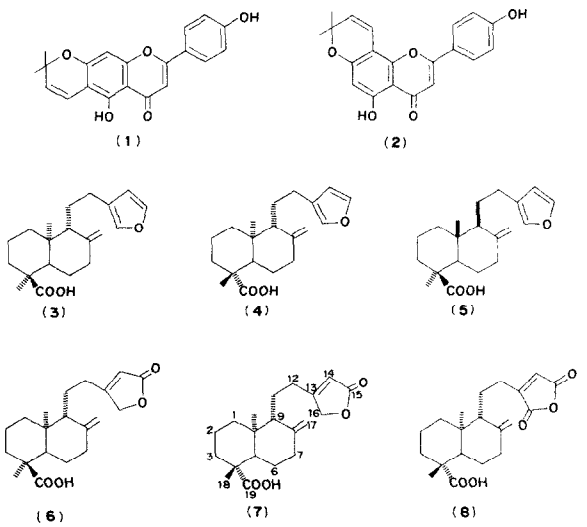
Chromatography of the plant extracts on silicic acid and workup of those fractions eluted with chloroform–ethyl acetate gave small amounts of a new flavone which proved to be 4′, 5-dihydroxy-6″, 6″-dimethylpyrano (2″, 3″:7,6) flavone (**1**). The flavone was a dull yellow product, mp 276–278°. Its UV spectrum resembled in profile that of a flavone, but did not correspond in detail with that of any representatives in several catalogues of flavonoid UV spectra [4,5]. The UV spectrum had the usual long wavelength 330 nm band common to many flavones, with extensive tailing towards longer wavelengths, but in addition, had a band at 310 nm. Nevertheless, the UV spectrum showed some features in common with apigenin. The flavone gave an aluminum chloride shift indicating the presence of a 5-hydroxy group [4]. The flavone gave no sodium acetate or boric acid shifts, indicating the absence of a 7-hydroxy and *o*-dihydroxy groups [4].

The flavone upon acetylation formed a diacetate. NMR studies were carried out on the diacetate due to its better solubility in convenient NMR solvents. The NMR spectrum of the diacetate did not show any methoxy resonances. It showed two AB doublets in the aromatic and vinyl region, two aromatic singlets, two acetyl resonances and a six proton C-methyl resonance. The slight downfield position of one of the acetoxy resonances suggested it was located at the 5-position. This was confirmed by the benzene solvent shift in which one of the acetoxy did not move upfield in benzene. This resonance must be due to a 5-acetoxy group. Acetoxy resonances normally shift 20–30 Hz upfield in benzene compared to their position in $CHCl_3$. This applies to acetoxy groups at all positions in flavones except those at the 5-position which do not show an upfield shift in C_6H_6 [6].

The set of AB doublets with 10 Hz coupling constants along with the 6-proton C-methyl resonance suggested the presence of a 2,2-dimethyl benzochromeme system in the flavone [7]. The presence of a 4′-hydroxy substituted B-ring suggested that the dimethylchromene ring must necessarily be located on the A-ring. Two possible orientations (**1** and **2**) of the benzochromene ring are thus possible. The linear possibility (**1**) would leave H-3 and H-8 as isolated protons giving rise to two singlets in the NMR spectrum.

* Part 11 in the series "Chemotaxonomy of the Rutaceae". For Part 10 see Dreyer, D. L. and Huey, P. F. (1974) *Phytochemistry* **13**, 1239.

The second, angular, possibility (**2**) would leave H-3 and H-6 as isolated protons, again giving rise to two singlets in the NMR spectrum.



Since H-3, H-6 and H-8 protons in flavone systems come at overlapping ranges in the NMR spectrum [8], it is not possible to distinguish between structures **1** and **2** by simple NMR methods. The Gibb's test [9] indicates unsubstituted para hydroxy positions. When carried out on the flavone, a positive test was obtained, indicating that the pigment does not have a group in the 8-position and thus must have structure **1** [10]. The MS was consistent with the gross structure. Thus, loss of methyl, M-15, gave rise to the base peak. This was supported by a metastable ion at *m/e* 306·7. The C-ring fragmentation frequently found with simple flavones gave rise to the fragments causing *m/e* 203 and 118 peaks.

C-Isopentenylation is a common process in the Rutaceae in coumarins, acridone and quinoline alkaloids. Indeed, the basis of the biosynthesis of the frequently occurring furocoumarins [11] and furoquinoline alkaloids [12] involves *C*-isopentylation. There is only one example of a *C*-isopentyl substituted flavonoid heretofore found in the Rutaceae; 8-isopentylflavanones in *Phellodendron japonicum* [13]. Isopentenyl substituents, when found in flavonoids are more generally associated with isoflavones occurring in the Leguminosae [14] and flavones of the Moraceae [15].

In an attempt to isolate additional amounts of the flavone, plant material from the following season was extracted and chromatographed with quite different results. Upon standing, the initial fractions from the column, eluted with hexane, deposited a crystalline product, mp 124·5–126·5°. The IR spectrum, with a single carbonyl band at 1692 cm^{-1}, suggested that in spite of the nonpolar nature of the compound, it was an acid. The UV spectrum showed only end adsorption. The NMR spectrum suggested a total of 28 protons with two C-methyl groups. When considered with the MS which showed a molecular ion at *m/e* 316, a C_{20} diterpene was indicated. The compound showed five protons in the vinyl-aromatic region. It gave a positive Ehrlich's test indicating the presence of a furan ring. The NMR spectrum further showed that the furan ring must be β-substituted.

Three C_{20} stereoisomers have been found in nature which would correspond in their gross structural features to that indicated by the spectroscopic data. These are polyalthic acid (**3**) [16], daniellic acid (**4**) [17] and lambertianic acid (**5**) [18]. Of the three known possibilities only the physical properties of daniellic acid (**4**) compares well with those found for the diterpene in this study. Comparison with an authentic sample of **5** indicated identity except for opposite sign of rotation so that the furoditerpene must be daniellic acid (**4**). Fractions eluted from the column contained a second diterpene. On the basis of its NMR spectrum, the second diterpene appeared to be closely related to **4**. The MS indicated a MW of 332; the IR spectrum indicated that it too was an acid, but an additional intense band at 1745 cm indicated the presence of a carbonyl group. The NMR spectrum showed again the presence of an exocyclic double bond, but contained an additional one-proton vinyl singlet and a two-proton downfield singlet at δ 4·72. The chemical shift of the vinyl singlet at δ 5·83 suggested the presence of β-substituted-α,β-unsaturated carbonyl system. The presence of this chromophore was supported by a λ_{max} at 216 nm [19] and a positive Raymond test [20]. The sum of the spectroscopic data indicated a butenolide structure. A compound of structure **6** has been reported by Henrick and Jefferies [21] but proved to be non-identical with the *Pamburus* diterpene.

However, it is reasonable that the two diterpenes found in this study should be stereochemically homogenous since they both came from the same plant. The close similarity of the *C*-methyl resonances of the butenolide with those of daniellic acid suggest that they do have the same relative stereochemistry. The butenolid is thus formulated as eperu-8(20)-en-15,19-dioic acid butenolide (**7**). Attempted correlation of **7** with **4** by oxidation of **7** to the maleic anhydride (**8**) with Jones reagent was unsuccessful. The enantiomer of **8** has previously been prepared from **5** by Jones oxidation [18].

The isolation of diterpenes from *Pamburus* is surprising since diterpenes are not common in the Rutaceae. Only one previous example has been reported from this well studied family [22]. These diterpenes may well have been present in the first batch of plant material and have been overlooked due to their initial reluctance to crystallize.

EXPERIMENTAL

NMR spectra were taken at 100 MHz. The areas of the signals were consistent with their assignments.

Isolation. Foliage of *Pamburus missionis*, obtained from the U.S. Plant Introduction Station, Coral Gables, Florida was dried, ground and extracted with C_6H_6. Solvent was removed from the extracts and the residue chromatographed on silicic acid. The column was eluted successively with more polar solvents. The fractions from the column were monitored by TLC. None of the fractions showed any striking fluorescence when viewed in UV light. Fractions eluted with $CHCl_3$–EtOAc deposited yellow crystals of flavone (**1**), mp 276–278°, from EtOAc; black $FeCl_3$ test; λ_{max}^{EtOH} 236, 273, 279, 313, 330, ~355 nm; $\lambda_{max}^{EtOH-NaOH}$ 402 nm; $\lambda_{max}^{EtOH-AlCl_3}$ 352, 410 nm; λ_{max} 690 nm in the Gibbs test; MS *m/e* (%), 336 (18), 322 (20), 321 (100), 203 (45), 18 (20). Found: M^+ 336·0989. $C_{20}H_{16}O_5$ requires: M^+ 336·0998. Found: M^+-15 321·0767. $C_{19}H_{15}O_5$ requires: 321·0763. The diacetate crystallized from MeOH, mp 216–219°; λ_{max}^{EtOH} 228, 283, 322 nm; NMR δ 7·56 (*d*, *J* 8) H-2′ and H-6′, 6·96 (*d*, *J* 8) H-3′ and H-5′, 6·58 (*d*, *J* 10) 6·28, 6·24 (*s*) H-3 and H-8, 5·49 (*d*, *J* 10), 2·32, 2·23 acetoxys, 1·44 C-methyls ($CDCl_3$); δ 2·23, 1·88 acetoxys, 1·26 *C*-methyls (C_6H_6).

Plant material from the subsequent season was worked up as described above and the following diterpenes were obtained: daniellic acid (**4**), mp 127–128·5° (C_6H_6–hexane); $[\alpha]_D$ −56° ($CDCl_3$); *v* 1692/cm (Nujol); NMR δ 7·30, 7·16 α-furan, 6·16 β-furan, 4·88, 4·57 vinyl, 1·21, 0·58 C-methyls ($CDCl_3$); MS *m/e* (%) 316 (100), 189 (25), 168 (10), 150 (15), 149 (10), 148 (14), 140 (14), 136 (9), 134 (13), 122 (34), 110 (9), 108 (16), 95 (17), 94 (15), 93 (9), 91 (18), 82 (35), 81 (37) (Found: C, 76·16; H, 9·14. $C_{20}H_{28}O_3$ requires: C, 75·91; H, 8·92%). Daniellic acid methyl ester prepared with diazomethane; non-cryst.; NMR δ 7·29, 7·14 α-furan, 6·18 β-furan, 4·89, 4·59 vinyl, 3·58 methoxy, 1·14, 0·50 C-methyls (CCl_4). Eperu-8(20)-en-15,19-dioic acid butenolide **7**; mp 145–147° (MeOH); $[\alpha]_D$ −49° ($CDCl_3$); *v* 1745, 1695, 1635/cm (Nujol); λ_{max}^{EtOH} 216 nm; NMR δ 5·83 (*s*), H-14, 4·90, 4·50 vinyl, 4·72, H-16, 1·23 0·62 C-methyls ($CDCl_3$); MS *m/e* (%) 332 (2), 235 (43), 217 (13), 189 (56), 133 (10), 121 (22), 109 (10), 107 (13), 105 (10), 98 (100), 95 (10), 93 (13), 91 (14), 81 (62), 79 (12), 67 (13), 55 (14), 41 (20), 39 (10) (Found: C, 72·33; H, 8·49. $C_{20}H_{28}O_4$ requires: C, 72·24; H, 8·49%).

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